

Historic, Archive Document

Do not assume content reflects current scientific knowledge, policies, or practices.

2521
.A75467
c3

Molting Inhibitory Effects of Azadirachtin on Large Milkweed Bug

U.S. Department of Agriculture
Science and Education Administration
Agricultural Research Results ARR-NE-5 October 1979

Mention of a commercial or proprietary product in this report is solely to provide specific information. It does not constitute endorsement by the U.S. Department of Agriculture over other products not mentioned.

A free copy of this publication is available from the Livestock Insects Laboratory, Beltsville Agricultural Research Center-East, Beltsville, Md. 20705.

Science and Education Administration, Agricultural Research Results,
Northeastern Series, No. 5, October 1979

Published by Agricultural Research (Northeastern Region), Science and Education Administration, U.S. Department of Agriculture, Beltsville, Md. 20705

MOLTING INHIBITORY EFFECTS OF AZADIRACTIN

ON LARGE MILKWEED BUG

By R. E. Redfern, J. D. Warthen, Jr., G. D. Mills, Jr., and E. C. Uebel^{1/}

ABSTRACT

Molting was severely inhibited when two kernel fractions of neem (Azadirachta indica A. Juss) were topically applied to the newly molted fifth-stage large milkweed bug (Oncopeltus fasciatus (Dallas)). The active component of these neem fractions was azadirachtin. Complete molting inhibition occurred when the nymphs were treated topically with as little azadirachtin as 0.355 µg per insect; all insects died before completing the nymph-to-adult molt.

KEYWORDS: Azadirachtin in neem fractions, insect growth regulator, large milkweed bug, molting inhibitors on Oncopeltus fasciatus, Oncopeltus fasciatus.

INTRODUCTION

The kernels of the neem tree (Azadirachta indica A. Juss)^{2/} contain a substance that has been shown to deter feeding in several insect species (Ruscoe, 5; Nakanishi, 3; Zanno et al., 9; Meisner et al., 2; Radwanski, 4; Warthen et al., 7, 8; Warthen, 6).^{3/} Azadirachtin markedly affected certain caterpillars and especially the cotton stainer (Dysdercus suturellus (Herrich-Schäffer)). The treated insects developed much slower than the controls (Ruscoe, 5). Leuschner (1) extracted leaves of A. indica with methanol and topically applied the extract to fifth-stage nymphs of a shield bug, Antestiopsis orbitalis bechuana (Kirk). The extract had morphological effects, and a postulation was made that the activity might be due to an ecdysone analog.

^{1/}R. E. Redfern and G. D. Mills, Jr., Livestock Insects Laboratory (formerly Biological Evaluation of Chemicals Laboratory), and J. D. Warthen, Jr., and E. C. Uebel, Biologically Active Natural Products Laboratory, Beltsville Agricultural Research Center-East, Beltsville, Md. 20705.

^{2/}Also referred to as Melia azadirachta L., M. indica Brandis, margosa tree, or Indian lilac.

^{3/}Underlined numbers in parentheses refer to Literature Cited at the end of this report.

The purpose of this study was to determine the effects of azadirachtin on the morphological development of the large milkweed bug (Oncopeltus fasciatus (Dallas)).

MATERIALS AND METHODS

Large milkweed bugs (newly molted fifth stage), reared in a 9.5- by 17- by 30-cm plastic sweater box at $27^{\circ} \pm 1^{\circ}$ C and 50 ± 5 percent relative humidity, were collected from our laboratory stock culture.

An authentic sample of azadirachtin, obtained from K. Nakanishi of Columbia University, New York, was used without further purification as a standard (fig. 1). The combined numbered fractions (table 1) were by hplc^{4/} recycling of A-16, which in turn was from 18.0 mg A (fig. 2) according to Warthen et al. (7). Some fractions were combined and others discarded because of the recycle procedure and the retention volume of the molting inhibitor. The samples were dissolved in acetone at 5 μ g equivalents per 1 μ l or 5 μ g per 1 μ l for sesamex. Each insect received 1 μ l of the acetone solution applied topically to the last three ventral abdominal segments with a calibrated 1- μ l glass micropipet. Treated insects (five per treatment) were confined in a $\frac{1}{2}$ -pint ice cream carton bottom fitted with a clear plastic petri-dish lid. They were furnished milkweed seed and water in cotton-stoppered shell vials. Treated nymphs were held for 7 days and then observed for mortality or morphological changes associated with chitin inhibition or juvenile hormone (JH) action. The JH rating system for O. fasciatus is as follows:

- 0 = perfect adult, no JH activity
- 1 = perfect adult, except retention of nymphal coloration in abdomen
- 2 = adult with reduced wings and nymphal coloration in abdomen
- 3 = second nymph, supernumerary nymph

^{4/}High performance liquid chromatography.

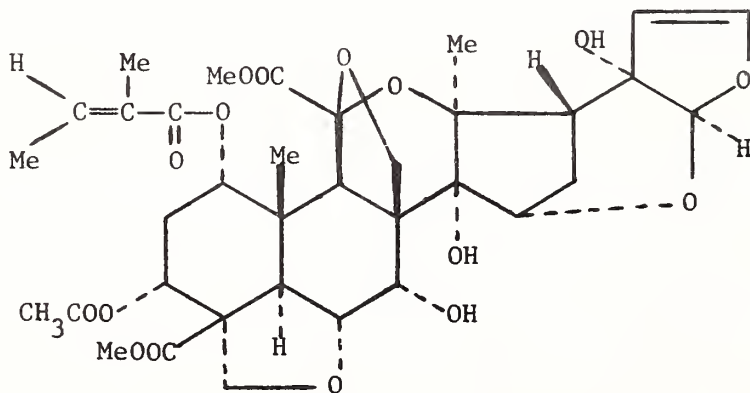


Figure 1.--Azadirachtin (Zanno et al., 9).

Table 1.--Bioassay for molting inhibiting activity against 5th-stage *Oncopeltus fasciatus* nymphs of fractions from hplc recycling of A-16 and authentic azadirachtin^{1/}

Fraction ^{2/}	Rate per nymph	Actual rate per nymph	Average results
	<u>µg equivalents</u>	<u>µg</u>	
1-14-----	10	---	Negative (normal adult).
15-----	10	---	Do.
18-----	10	---	Do.
19-31-----	10	---	1.0 JH. ^{3/}
32-34-----	10	---	100 percent mortality. ^{4/}
	5	---	Do.
	2.5	---	1.0 JH.
51-57-----	10	0.71	100 percent mortality.
	5	.355	Do.
	2.5	.1775	Do.
	1.25	.08875	0.5 JH.
Azadirachtin (standard) ^{5/}	10	.71	100 percent mortality.
	5	.355	Do.
	2.5	.1775	1.6 JH; 40 percent mortality.
	1.25	.08875	1.0 JH.
Sesamex (standard)	-----	10	3.0 JH.
		1	.6 JH.
		.1	0 JH.
Acetone (check 1 µl)-----	-----	---	Negative (normal adult).

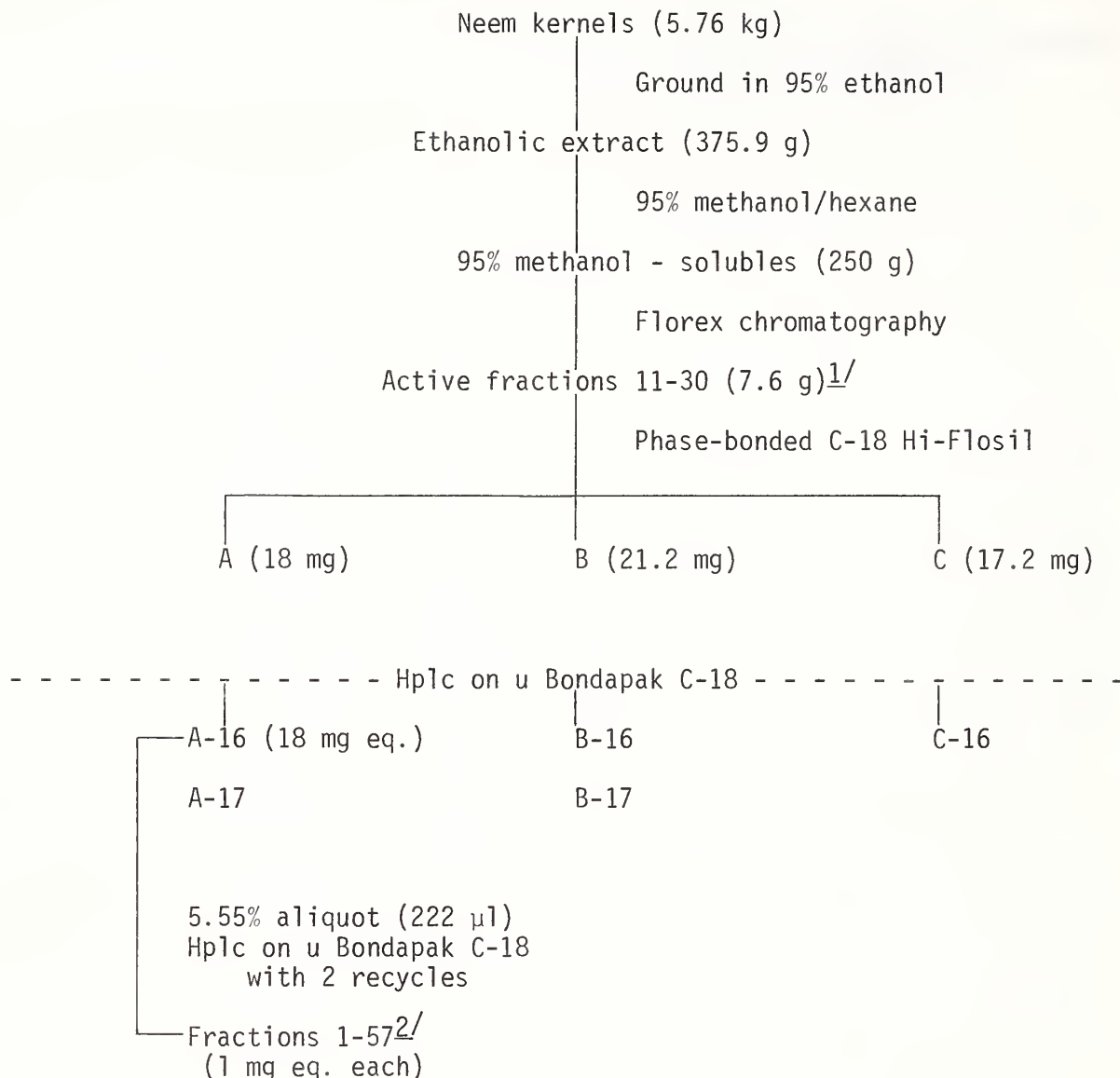
^{1/} 3 replicates of 5 nymphs for each test.

^{2/} Each numbered fraction contains 1 mg equivalent from 222-µl hplc injection of A-16 (18 mg eq. per 4 ml) (Warthen et al., 8).

^{3/} For description of JH effects, see text.

^{4/} Unable to complete molt-to-adult stage applies to each percent result.

^{5/} 0.25 mg eq. 17.75 µg (Warthen et al., 8).



^{1/} All resulting A, B, and C fractions are active.

^{2/} See table 1.

Figure 2.--Flow diagram of azadirachtin isolation from neem kernels.

RESULTS AND DISCUSSION

The effects of topically treated newly molted fifth-stage nymphs of *O. fasciatus* are shown in table 1. Combined fraction 1-14 and fractions 15 and 18 showed no activity; combined fraction 19-31 showed no molting inhibiting activity; however, it did produce slight JH abnormalities. Combined fractions 32-34 and 51-57 resulted in strong molting inhibiting activity at 10 and 5 µg

equivalents and 10, 5, and 2.5 μ g equivalents, respectively, and both produced slight JH activity at the lower dosages. The azadirachtin standard gave less molting and more JH activity than the combined fraction 51-57 that contained azadirachtin.

The amount of azadirachtin had already been calculated for the combined fraction 51-57 by ultraviolet absorption (Warthen et al., 7). One can thus see that complete molting inhibition occurred when the nymphs were treated topically with as little azadirachtin as 0.355 μ g per nymph; all insects died before completing the nymph-to-adult molt.

LITERATURE CITED

- (1) Leuschner, K. 1972. Effects of an unknown plant substance on a shield bug. *Naturwissenschaften* 59: 217-218.
- (2) Meisner, J., M. Wysoki, and K. R. S. Ascher. 1976. The residual effects of some products from neem (Azadirachta indica A. Juss) seeds upon larvae of Boarmia (Ascotus) selenaria Schiff, in laboratory trials. *Phytoparasitica* 4: 185-192.
- (3) Nakanishi, K. 1975. Structure of the insect antifeedant azadirachtin. *Recent Advn. Phytochem.* 9: 283-298.
- (4) Radwanski, S. 1977. Neem tree 3: Further uses and potential uses. *World Crops and Livestock* 29: 167-168.
- (5) Ruscoe, C. N. E. 1972. Growth disruption effects of an insect antifeedant. *Nature (New Biol.)* 236: 159-160.
- (6) Warthen, Jr., J. D. 1979. Azadirachta indica: A source of insect feeding inhibitors and growth regulators. U.S. Dept. Agr., Sci. and Educ. Admin., Agr. Rev. and Manuals, Northeastern Ser., No. 4, 21 pp.
- (7) Warthen, Jr., J. D., R. E. Redfern, E. C. Uebel, and G. D. Mills, Jr. 1978. An antifeedant for fall armyworm larvae from neem seeds. U.S. Dept. Agr., Sci. and Educ. Admin., Agr. Res. Results, Northeastern Ser., No. 1, 9 pp.
- (8) Warthen, Jr., J. D., E. C. Uebel, S. R. Dutky, and others. 1978. Adult house fly feeding deterrent from neem seeds. U.S. Dept. Agr., Sci. and Educ. Admin., Agr. Res. Results, Northeastern Ser., No. 2, 11 pp.
- (9) Zanno, P. R., I. Miura, K. Nakanishi, and D. L. Elder. 1975. Structure of the insect phagorepellent azadirachtin. Applications of PRFT/CWD carbon-13 nuclear magnetic resonance. *J. Amer. Chem. Soc.* 97: 1975-1977.

U. S. DEPARTMENT OF AGRICULTURE
SCIENCE AND EDUCATION ADMINISTRATION
NORTHEASTERN REGION
AGRICULTURAL RESEARCH CENTER WEST
BELTSVILLE, MARYLAND 20705

OFFICIAL BUSINESS
PENALTY FOR PRIVATE USE, \$300

POSTAGE AND FEES PAID
U. S. DEPARTMENT OF
AGRICULTURE
AGR 101



9606 USSER1533A122 03001 0001
USDA SEA RIO
BOX 53326
NEW ORLEANS LA 70153